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# MICROBIOLOGICAL QUALITY ASSESSMENT OF EXPOSED *GARRI* SOLD IN SELECTED MARKETS IN OYE-EKITI, NIGERIA

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## ABSTRACT

*A common Nigerian fermented staple meal, garri can become contaminated during manufacturing, marketing and storage by a variety of germs, which could be harmful to consumers' health. In this work, isolates from garri sold in Oye-Ekiti Local Government Area were phenotypically and molecularly characterized. Nine samples were purchased from three different markets using sterile paper bags and examined in the laboratory. Based on morphological characteristics and biochemical tests, the organisms isolated and their frequency of occurrence were Staphylococcus spp. (15 %), Bacillus spp. (70 %) and Enterobacter spp. (15 %) and Aspergillus spp. (100 %). Molecular characterization of two distinct Bacillus spp. identified them as Bacillus subtilis and Brevibacillus brevis exhibiting 85.9 % and 98.8 % percentage similarities, respectively. In order to minimize the level of contamination of garri by hazardous microorganisms, local processors of the product should be trained and retrained on food handling, food packaging, sanitation and hygienic practices.*

**Keywords:** *Garri*, Bacteria, Cassava, fermented food, molecular characterization.

## INTRODUCTION

Cassava (*Manihot esculenta*) is the fifth most grown plant in the world and the main source of nutrition for almost 500 million people worldwide, making it an essential staple in underdeveloped countries including Nigeria (Olaoye *et al.*, 2015). Spontaneous fermentation of cassava is common in resource-poor settings; it helps transform harvested tubers into more durable food products while lowering cyanide levels and improving nutritional content. Additionally, fermentation improves flavour, aroma, and colour of a food product, which increases customer satisfaction (Sunday *et al.*, 2023). Cassava is the main raw material used in the traditional and essential West African dish especially *garri.* Indigenous knowledge of *garri* processing practiced for many centuries result in varied quality of the product in the markets depending on the producer. It is a source of nutrients sustainable for many rural communities (Bayode and Ojokoh, 2020).

The process of producing *garri* involves peeling, cleaning, and grating the cassava roots. Thereafter, the grated mash is pressed, sieved, fermented (if desired), and roasted to drastically reduce the level of water in the product. The classification of *garri* in Nigeria typically referred to as "*Ijebu garri*" and "yellow *garri*". All types of *garri* including 'yellow *garri*' undergo fermentation to some

extent. It is a known process that reduces the level of cyanide to a safe level in the product. While yellow *garri* is made without fermentation but is made by adding red palm oil before roasting, *Ijebu garri* is made by fermenting the cassava mash (Erukainure*et al*., 2022). *Garri* is eaten in different ways in Nigeria, including as a sticky paste called *eba*, soaked in cold water, and combined with other meals like *moi-moi*and porridge beans. Since the total cyanide concentration of *garri*is reduced as a result of various processing methods, it may not pose a serious health risk to consumers. (Adesemoye *et al*., 2021).

Several communities in West African have a strong cultural bond with *garri* production, and consuming it goes beyond simple nourishment to include social and ceremonial occasions. Moreover, *garri* is essential to the local economies of many West African nations, creating jobs at every stage of the value chain from cassava farming to *garri* selling and processing (Olaoye *et al*., 2015). Processing conditions, handling, storage containers and prolonged exposure of *garri* to the environment during sales increase the risk of microbial contamination of *garri.* (Ogunbodede and Ogu, 2019). Foodborne illnesses can arise due to consumption of spoilt foods degraded by microorganisms. Molds, insects and mice are mainly responsible for the spoilage of *garri.* During storage and distribution of *garri* especially in humid conditions, molds are most likely to cause spoilage of the product.

The growth of molds and other microorganisms in *garri* could affect the microbiological, organoleptic, and nutritional qualities of *garri* (Adesemoye *et al*., 2021).

Various microbial species, including *Staphylococcus aureus*, *Escherichia coli, Salmonella* sp., *Shigella* sp., *Aspergillus* sp., *Cladosporium* sp., and *Fusarium* sp., have been identified in stored, retailed, and ready-to-eat garri from specific Nigerian communities (Ogbonna *et al*., 2017; Okafor and Mmaduabuchi, 2022). These microorganisms pose public health risks, underscoring the crucial need to uphold quality and safety standards in *garri* production (Okolo and Makanjuola, 2021). Despite several reports on microbial contamination of *garri*, it continues to be a food of choice in numerous households and communities, often consumed without any pre-treatment or cooking. This practice may potentially expose consumers to health risks associated with microbial contamination and their toxins. The threats these microorganisms represent to public health highlight how important it is to maintain quality and safety standards during the processing of *garri* (Okolo and Makanjuola, 2021). Hence this study is intended to isolate and identify organisms found in exposed *garri* sold in some markets in *Oye Ekiti* using both traditional and molecular methods

## MATERIALS AND METHODS

### Study area

This research was taken place within the *Oye* Local Government area of Ekiti State, Nigeria. Oye Ekiti local government area is one of the 16 local government areas in Ekiti State, Nigeria. It is situated in the Northern part of Ekiti State. The major towns located in Oye Ekiti local government area are Oye, Ayegbaju, Itapa, Ilupeju, Ire, Ijelu and Ayede. Open markets from different towns in Oye Local government (*Oye, Aiyegbaju* and *Ilupeju*) were randomly selected for this study

### Sample collection

Three samples 110g each of white *garri* were obtained from three vendors in each of the market and the control was collected immediately after *garri* was roasted. A composite sample of *garri* in a basin displayed in the market by each vendor was collected from the top, middle and bottom.

The samples were collected using sterile polythene bag and transported to the laboratory for immediate analysis. The samples were labeled as follows OA, OB, OC

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(Oye market), IA, IB, IC (Ilupeju market), AA, AB AC (Aiyegbaju market), and C (control).

**Sample analysis**

In a test tube, one gram of each sample was added to nine milliliters of sterile distilled water, and the mixture was allowed to stand for five minutes. The tube was thoroughly mixed, and 1 ml from the stock was added to the second test tube, which contained 9 ml of sterile distilled water, using a sterile syringe. This process was repeated until the final dilution using a sterile syringe for each transfer. This procedure was repeated for all the samples.

One milliliter of the diluted sample from the $10^{-2}$ and $10^{-4}$diluent was placed on nutrient agar (NA), MacConkey agar and streptomycin-supplemented potato dextrose agar (PDA) plates using the pour plate method. While the PDA plates for isolation of fungi were kept at room temperature (28±1°C) for 48–72 hours, the NA and MacConkey plates for isolation of bacteria were incubated at 37°C for 18–24 hours. The colonies were enumerated with the aid of colony counter and converted to CFU/g. Distinct colonies were sub cultured on fresh agar plates until pure cultures was obtained. Throughout the period biochemical tests, were carried out on the discrete colonies inoculated in slants using appropriate media and maintained at 4°C.

**Phenotypic characterization**

The pure isolates were identified based on their colonial and cultural characteristics, cellular traits (shape and Gram staining), and biochemical tests. Bergey's Manual of Determinative Bacteriology was used as a reference for identification (Cheesbrough, 2006). Fungal isolates were identified based on their morphological features. Slides of the fungi was prepared using cotton blue lactophenol, and these slides were examined under a microscope using x10 and x40 objectives. Tentative identification of the isolates was carried out using the Fungi atlas (Ogbonna*et al*., 2021).

**DNA extraction**

First, 100 mg of resuspended bacterial cells and 750 µL of Lysis Solution were added to a ZRBashingTMLysis Tube. After being fastened, the tube was processed for more than five minutes at maximum speed in a bead-beating device. The tube was centrifuged at more than 10,000 x g for one minute following processing. A Zymo-Spin IV Spin Filter in a Collection Tube was filled with up to 400 µL of the supernatant, and the tube was centrifuged at 7,000 x g for a minute. Subsequently, the filtrate in the Collection Tube was mixed with 1,200 µL of Bacterial DNA Binding Buffer. The mixture was then moved to a Zymo-SpinTMIIC Column in 800 µL aliquots, and it was centrifuged at 10,000 x g for one minute, discarding the flow-through after each centrifugation. A 200 µL DNA Pre-Wash Buffer was added to the column in a fresh Collection Tube, and it was centrifuged at 10,000 x g for a minute. Next, 500 µL of Bacterial DNA Wash Buffer was added, and it was centrifuged again. Ultimately, 100 µL DNA Elution Buffer was added to the column matrix after the Zymo-SpinTMIIC Column was moved to a sterile 1.5 mL microcentrifuge tube. After the tube was centrifuged for 30 seconds at 10,000 x g to elute the DNA, it was prepared for PCR.

**Amplification of the rRNA gene**

The PCR reaction was prepared by combining 2.5 µL of 10x PCR buffer, 1 µL of 25 mMMgCl$_2$, 1 µL each of forward and reverse primers, 1 µL of DMSO, 2 µL of 2.5 mM dNTPs, 0.1 µL of 5 U/µL Taq DNA polymerase, and 3 µL of 10 ng/µL DNA. The total reaction volume was adjusted to 25 µL with 13.4 µL of nuclease-free water. The primer sequences used were 27F (AGAGTTTGATCCTGGCTCAG) and 1525R (AAGGAGGTGWTCCARCCGCA). The PCR program involved an initial denaturation at 94˚C for 5 minutes, followed by 36 cycles of denaturation at 94˚C for 30 seconds, annealing at 56˚C for 30 seconds, and elongation at 72˚C for 45 seconds. This was followed by a final elongation step at 72˚C for

7 minutes, with the temperature then held at 10˚C indefinitely. The amplified fragments, approximately 1500 bp in size, were visualized on ethidium bromide-stained 1.5% agarose gels, using a 1 kbp DNA ladder for size reference. The ABI 3500 instrument was utilized for the sequencing process. For the cycle sequencing, a 96 well plate was employed, and the resulting products underwent purification using the Ethanol/EDTA precipitation method. A quantity of 25ng from the PCR product was utilized for the cycle sequencing step. Using the Basic Local Alignment Search Tool (BLAST, http://www.ncbi.nlm.nih.gov/BLAST), the strains were found by homology search.

## RESULTS AND DISCUSSION

### Results

**Table 1: Bacterial and coliform count (CFU/g) of *garri* samples**

| Sample | Bacteria count | Coliform count |
|---|---|---|
| **OA** | $1.5\times10^{4}$ | $1.5\times10^{2}$ |
| **OB** | $1.6\times10^{4}$ | $1.6\times10^{3}$ |
| **OC** | $1.2\times10^{4}$ | $1.2\times10^{2}$ |
| **IA** | $1.2\times10^{4}$ | $1.2\times10^{3}$ |
| **IB** | $6.4\times10^{3}$ | $6.4\times10^{1}$ |
| **IC** | $7.3\times10^{3}$ | $7.3\times10^{2}$ |
| **AA** | $6.3\times10^{3}$ | $6.3\times10^{1}$ |
| **AB** | $1.4\times10^{3}$ | $1.4\times10^{3}$ |
| **AC** | $1.9\times10^{3}$ | $1.9\times10^{2}$ |
| **C** | $5.2\times 10^{3}$ | $1.1\times10^{1}$ |

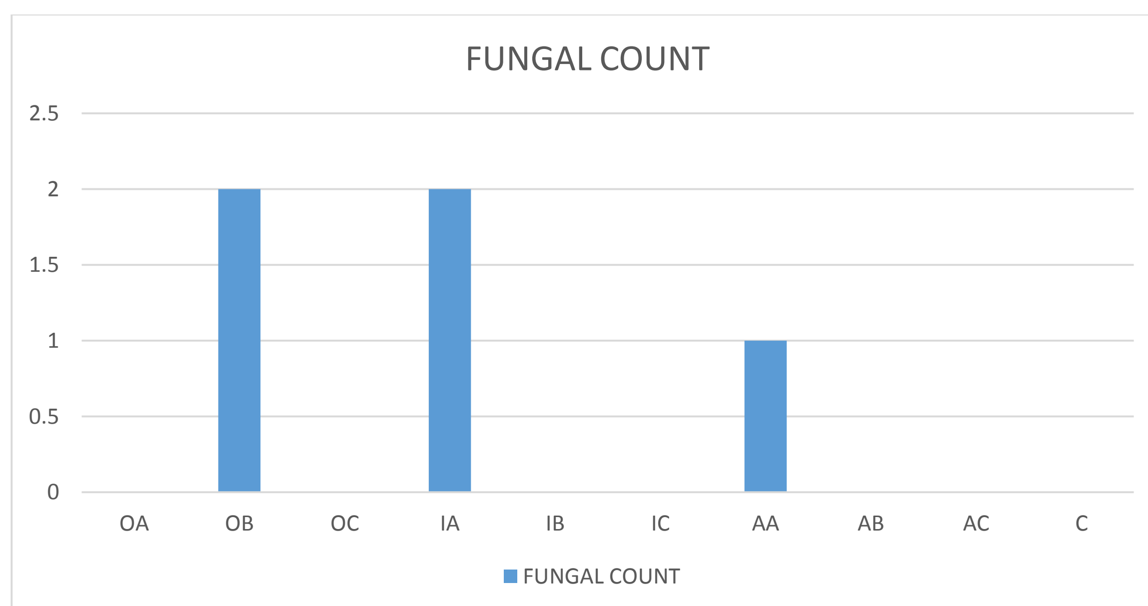


**Figure 1: Bar chart showing the fungal count of *garri*samples**

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**Table 2 Phenotypic characterization of the bacteria isolates**.

| Isolates codes | Cell morphology | Gram reaction | Catalase | Oxidase | Urease | Indole | Starch hydrolysis | MRVP | Sucrose | Lactose | Mannitol | Glucose | Fructose | Probable organism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1C1** | Rod | + | + | + | - | - | + | - | - | - | + | - | + | *Bacillus* spp. |
| **AA2** | Rod | + | + | + | - | - | + | + | + | - | - | + | + | *Bacillus* spp. |
| **AC3** | Rod | - | + | + | - | - | - | - | - | - | + | - | - | *Enterobacter* sp. |
| **C4** | Short rod | + | + | + | - | - | + | + | + | - | - | + | + | *Bacillus* spp. |
| **IB1** | Short rod | + | + | + | - | - | + | - | - | - | + | - | + | *Bacillus* spp. |
| **OA2** | Rod | + | + | + | - | - | + | + | + | - | - | - | + | *Bacillus* spp. |
| **OB2** | Rod | + | + | + | - | - | - | - | - | - | + | - | - | *Bacillus* spp. |
| **OC2** | Cocci | + | + | + | - | - | + | - | + | - | + | + | + | *Staphylococcus* sp. |

**Key: + positive, - negative, MRVP Methyl Red- Voges- Proskauer**

**Table 3. Phenotypic identification of Fungi Isolates**

| Isolate code | Probable organism |
|---|---|
| **AA1** | *Aspergillus flavus* |
| **IA1** | *Aspergillus flavus* |
| **IA2** | *Aspergillus niger* |
| **OB1** | *Aspergillus nidulans* |
| **OB2** | *Aspergillus niger* |

**Table 4: molecular characterization of two randomly selected isolates**

| Sample code | Presumptive organism isolated | Molecular Identification | Strains | Percentage similarity |
|---|---|---|---|---|
| **IC1** | *Bacillus* sp. | *Bacillus subtilis* | **HM480326.1** | 86.2% |
| **OB2** | *Bacillus* sp. | *Brevibacillusbrevis* | **MG897037.1** | 98.8% |

## DISCUSSION

The total microbial counts from the examination of the *garri* samples obtained from three markets revealed that the fungal counts (0 CFU/g – $2.0 \times 10^1$ CFU/g) were lower than the total bacterial counts ($1.4 \times 10^3$ CFU/g – $1.6 \times 10^4$ CFU/g). The sample from *Oye* market had the highest microbial load ($1.2 \times 10^4$ CFU/g – $1.5 \times 10^4$ CFU/g). This may indicate increased bacterial contamination from the environment or during processing. As for the *garri* sold within a university campus in Zaria, Okolo and Makanjuola (2021) reported higher total fungal counts ($1.0 \times 10^3$ CFU/g – $2.0 \times 10^3$ CFU/g) and bacterial counts ($9.5 \times 10^3$ CFU/g – $6.7 \times 10^4$ CFU/g), Interestingly, this result is still within the tolerable limit of $10^4$ to $10^5$ recommended by the International Commission on Microbiological Specifications for Foods (ICMSF, 1996). However, coliform was found in all the *garri* samples at high levels, ranging from 6.3 $x10^1$CFU/g to 1.6 $x10^3$CFU/g, with samples from *Aiyegbaju* and *Oye* towns having the lowest and highest counts, respectively. This result is an indication that food safety and personal hygiene by food handlers were not adhered to unsanitary practices during post-process handling of *garri*

and the environment are possible sources of microbial contamination of *garri* displayed for consumers to buy the product in open markets. Ogunbodede and Ogu, (2019) also reported high coliform count in *garri* ($3.6\times10^2$ to $4.7\times 10^3$cfu/ml) from a local government in Enugu state which agrees with this study, on the contrary, Adesemoye *et al*., (2021) reported that no coliform was isolated from *garri* samples from Oye local government area. The ICMSF advised against the presence of coliforms in meals that are ready to eat like *garri*. All the samples are consequently of low quality and unfit for human consumption due to the presence of coliforms.

A number of human ailments could be associated with the presence of potential pathogenic bacteria found in *garri* usually consumed by susceptible groups like young children, the elderly, and people with weakened immune systems. *Enterobacter, Staphylococcus*, and *Bacillus* species were isolated from the *garri* samples. Poor hygienic standards during handling, processing, and retailing of could be responsible for contamination of the product.Being a commensal of the human microbiome, *Staphylococcus aureus* can contaminate food during processing if handled with bare hands. Its presence in *garri* samples indicates that contamination occurred either through direct touch or through mechanisms involving airborne droplets.

There is a public health risk as a result of *Aspergillus* spp. found in 100% of the *garri*samples obtained from the market. Some researchers have also reported the presence of *Aspergillus* spp. in *garri* samples (Ogbona*et al*., 2021; Okafor *et al*., 2022). Humans exposed to *Aspergillus* species can experience acute immunological and hepatotoxic effects as well as a high risk of having cancer during long-term exposure. Even when the best standards are followed, factors such food processing, storage, and transportation increase the likelihood of aflatoxins contamination of food (Ogbonna *et al*., 2021). Aflatoxins are associated with immunosuppression, decreased susceptibility to infections, stunted growth in children, and liver cancer, which accounts for 5% to 28% of instances of liver cancer. Aflatoxin-related cases have been linked to more than 70% of liver cancer that occurred in Nigeria in 2010 (Abt Associate, 2012).

Seventy-fivepercent of the isolated bacteria were identified as *Bacillus* species. Further characterization of the isolate using molecular methods revealed they were non-pathogenic (Table 4).The organisms were identified as *Bacillus subtilis* and *Brevibacillus brevis*, these organisms play a useful role in fermenting the cassava. Probiotic benefits such as enhanced gut health and pathogen suppression are associated with *Bacillus subtilis*, the organism is well known for its ability to produce spores necessary for its survival in harsh environmental conditions. Its ability to produce a variety of enzymes that breakdown complicated compounds has been reported (Zhang *et al*., 2020).Apart from its antibacterial property, *Brevibacillus brevis* also produces enzymes that help break down proteins, lipids, and carbohydrates (Lui *et al*., 2020). The properties of *Brevibacillus brevis* isolated from *garri* could confer health benefits to consumers, improve safety and quality of the product.

## CONCLUSION

In conclusion, the presence of coliforms and potential pathogenic organisms in *garri* is a source of concern despite the fact that the level of contamination is still within the acceptable limits of ICMSF. Hence, training and retraining of traditional producers of this fermented food on safety, hygiene and sanitation during production is of great importance. Also, routine microbiological examination of *garri* to ensure compliance with safety standards and early detection of potential contamination is needed.

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